Mammalian Genome 11, 871–876 (2000).
DOI: 10.1007/s003350010150

Mammalian Genome
Incorporating Mouse Genome

Comparative mapping of the ovine *clpg* locus

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Received: 3 February 2000 / Accepted: 19 April 2000

Abstract. We used a comparative mapping approach to identify segments of conserved synteny between human Chromosome 14 (HSA14), bovine Chromosome 21 (BTA21), and the portion of ovine Chromosome 18 (OAR18) that contains the *clpg* locus. A bovine radiation hybrid map of the region was constructed with available Type II genetic markers and seven candidate genes to establish the comparative interval between BTA21 and HSA14. We developed polymorphic microsatellite and SNP markers associated with five candidate genes and placed them on the ovine and/or bovine genetic maps by multipoint linkage analysis. *Three* additional genes were mapped by virtue of their physical linkage to genetically mapped makers. Development of integrated linkage and physical maps facilitates the selection of positional candidate genes from the gene rich human map. The physically linked candidate genes *PREF-1* and *MEG3* map to the interval containing the *clpg* locus. Comparative biology suggests imprinting of *MEG3* and/or the influences of *PREF-1* on cellular differentiation, should be examined for their role in the parent-of-origin dependent influence of mutant *clpg* alleles on sheep muscle characteristics.

Introduction

The existence of an ovine gene that influences sheep muscularity was first reported by Jackson and Green (1993). The genetic locus associated with the phenotypes has been named "callipyge" (*clpg*). Lambs expressing the callipyge phenotype are characterized by increased muscle accretion, decreased fat accretion, greater dressing percentage, and compact, lean carcasses relative to normal lamb phenotypes (Freking et al. 1998b). These are positive attributes in a meat production setting but are accompanied by a significant decrease in meat tenderness (Freking et al. 1999; Koohmaraie et al. 1995). Unique associations of *clpg* genotypes with phenotypes indicate epigenetic regulation of gene expression by parental imprinting (Cockett et al. 1996; Freking et al. 1998a; Georges and Cockett 1996). Only individuals receiving the mutant *clpg* allele from their sire and a wild-type allele from their dam exhibit the callipyge phenotype. All other genotypic combinations display normal carcass characteristics. Determination of the mechanism underlying the callipyge phenotype would improve our understanding of the epigenetic regulation of gene expression, as well as the biology of muscle development and physiology in sheep and other *Bovidae.* Ultimately this information could contribute to the development of management strategies aimed at efficient production of tender meat.

The *clpg* locus has been mapped to the distal arm of ovine Chr 18 (OAR18; Cockett et al. 1994), and its position subsequently refined to a 3.9-cM interval at 86 cM relative to the centromeric-most marker, insulin growth factor-1 receptor (*IGF-1R;* Freking et al. 1998a). OAR18 is orthologous to bovine Chr 21 (BTA21), as evidenced by the conservation of sequence content and order of many genetic markers (Cockett et al. 1994). The telomeric portion of human Chr 14 (HSA14) displays conserved synteny with the telomeric portions of BTA21 and OAR18 (Cockett et al. 1994; Solinas-Toldo et al. 1995). The q-arm of HSA14 is suspected of harboring maternally and paternally imprinted chromosomal regions, as revealed by uniparental disomy (Engel 1997), suggesting the possibility that expression of the human ortholog of the gene underlying the ovine *clpg* locus may also be subject to epigenetic regulation. The conservation of synteny between mammalian species provides an efficient way to establish boundaries of a conserved chromosomal segment containing a specific quantitative trait locus (QTL) by comparative mapping (Sonstegard et al. 1997). Defining livestock QTL intervals with type I markers allows the selection of positional candidate genes (Collins 1995) by comparison with orthologous regions of the highly characterized human genome.

Our objective was to establish a comparative map that precisely defines the orthology between the ovine chromosomal segment containing the *clpg* locus and HSA14q24.3-31.1. We have used a bovine radiation hybrid (RH) panel and bovine and ovine mapping populations to establish the boundaries of conserved synteny between sheep, cattle, and humans around the *clpg* locus. In addition, physical mapping data derived from genomic subclones that contain type I and type II markers further refined a map of this conserved segment. The functional candidate genes *PREF-1* (preadipocyte factor-1) *and MEG-3 (maternally expressed gene 3)* map to the same genetic interval as the *clpg* locus.

Materials and methods

PCR primers. Polymerase chain reaction (PCR) primer pairs (Tables 1 and 2) were designed by using Oligo 5.0 software MBI, Cascade CO) and synthesized on an Oligo 1000M oligonucleotide synthesizer (Beckman, Fullerton, Calif.). Amplification primers were designed from genomic sequence of cattle or sheep for *PAP, PREF-1,* and *AKT.* When bovine or ovine sequence was unavailable, as for *BDKRB2, EMAPL, YY1,* and *CKB*, primers were designed to match consensus vertebrate sequences. PCR amplification products for all loci were sequenced to verify identity and submitted to GenBank (Tables 1 and 2).

RH panel analysis. A 5000-rad bovine RH panel of 90 hybrids (BovR5; Womack et al. 1997) was screened by PCR amplification for the presence of type I and type II markers near the *clpg* locus. Marker linkage and order were determined with RHMAP3.0 software (Lunetta and Boehnke 1994).

Identification of genomic clones. To isolate new markers and develop a physical map near the *clpg* locus, large-insert genomic clones were screened for the presence of markers predicted to be in this region. The microsatellite marker OY15 was isolated from an ovine YAC (Broom and Hill 1994) that had previously provided markers near the *clpg* locus

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Table 1. DNA segments amplified and sequenced.

Gene or DNA segment	Sp.[a]	Symbol	PCR amplification primes (5′ → 3′)	GenBank Accession No.
Bradykinin receptor B2	B	*BDKRB2*	TTCTGGGCCATCACCATC CTCCTCTCCGTCTGGATCTC	AF207860
Bradykinin receptor B2 promoter	S	*BDKRB2*	ACATCACGACCCAGCCCTTGA CCTGCCTGCCTGTCTCCTCA	AF207861
Poly(A)polymerase	B	*PAP*	GGGTACATCGAGTGAAAGCAT TTTACCAAACGTGTTGAGGAA	X61585
Echinoderm microtubule associated protein-like	B	*EMAPL*	GCACACTGTCGGGGGACTTC GTGCCCACAGGTCAGGAACTG	AF207863
Echinoderm microtubule associated protein-like	S	*EMAPL*	GCACACTGTCGGGGGACTTC GTGCCCACAGGTCAGGAACTG	AF207864
Yin Yang 1 Transcription factor	B	*YY1*	GATATGCTTAGTAATGCTAC AATACAGCTATACGATACAAT	AF207865
Preadipocyte factor 1	B	*PREF-1*	CCATGGGCATCTCTTCCTCA GCCGGCCTCCTTGGTGAA	AH008244
Protein kinase B	B	*AKT*	CCAGATGATCACCATTACGC CAAACGCATCCAGAAATAAAAA	X61036
Brain creatine kinase	B	*CKB*	GGGGGCAACATGAAGGAGGTGT GGTTGGATGGGCAGGTGAGGAT	AF207866
OBDS564	S	OBDS564	GCTCCGAGGGCAGGGCTAA CCATCCAAGTCCCCAGTGAAGA	AF207868
OBDS567	S	OBDS567	CGCATCTCTATGGAACTCGTCA GATGCTAGGGCGTGGTCA	AF207869
OBDS562	S	OBDS562	CGTCTCCAGGCAAAAGGGTAGC ATCCCACATGAGCGAAAACAGC	AF207870
OBDS565	S	OBDS565	CCTGGCCTGATGGGGTAATGAC TCGCTTGTCTGGGCTGTGGAT	AF207871
OBDS566	S	OBDS566	CCTCCCACCACCTTGCCATCT TTTGAACTGCACAGCCCCTTGA	AF207872

[a]Species isolated from (Sp.). B = bovine, S = sheep.

Table 2. Polymorphic markers developed for linkage analysis.

Name	Marker no.	Marker type[a]	Number of alleles: Cattle	Sheep	Genotyping Primer (5′ → 3′)	Accession #
OY15	2243	MS	6	6	AGACAGCCTCTTTCCAGAACC GTCTTACCTGCTTCAGGGTCC	AF207867
AKT	2272	SNP	2	NA	CCAGATGATCACCATTACGC CAAACGCATCCAGAAATAAAAA	AF207874
AKT	2551	MS	7	NA	TGCCCATTCCCAGAGCCCTGT CAGCTCGCCCCAGGGTGG	AF207875
AKT	2697	MS	6	7	GCCGCTGGTTCCTCCTCCA CAGAGCCCTGCGTCCATCTTCT	AF224742
CKB	2576	MS	11	2	ACAGCAGGCCTGGAAGGTC CAGAGAGGGCTGCTTGTTCC	AF207878
BDKRB2	2355	MS	1	6	CTGCCCGATCCTTCTGCTT AAAGGGGCAGATTCAGTATCCA	AF207876
BDKRB2 promoter	2473	SNP	3	NA	ACATCACGACCCAGCCCTTGA CCTGCCTGCCTGTCTCCTCA	AF207862
PREF-1/MEG3	2535	MS	7	NA	GCAGAGCAGGTGCAGGACCCAC CACTCTCCTCTCCCTTA	AF207879
PREF-1/MEG3	2575	MS	NA	3	GAAGCCCCATACACAGAC TCCACACATGAAGTCACAA	AF207877
EMAPL	2412	SNP	2	NA	GCAACTAACACTTTCACTTTC TCCATCTGTTTCTCTTCCT	AF207863
EMAPL	2476	SNP	NA	2	GTGCCCACAGGTCAGGAACTG GGCGTCAGCTCAACAAGTCCA	AF207864

[a]MS = microsatellite, SNP = single nucleotide polymorphism, NA = not applicable.

(Freking et al. 1998a). An ovine BAC library (Gill et al. 2000) was screened by PCR for the presence of the microsatellite markers IDVGA-30 and BMS1561, previously mapped near the *clpg* locus (Freking et al. 1998a), as well as OY15. The isolated ovine BACs were end-sequenced for development of nonrepetitive markers (ovine BAC derived sequences, OBDS) suitable for both PCR amplification and DNA hybridization. BAC 317C4R6 contained OY15 and provided OBDS564 and OBDS567. BAC 34R8C2 contained IDVGA-30 and provided OBDS562 and OBDS565. BAC 56R3C11 contained BMS1561 and provided OBDS566. We also screened this library for a BAC containing the *BDKRB2* gene, to orient the meiotic map with respect to the human gene map (Deloukas et al. 1998) resulting in the isolation of a single ovine BAC, 101C12R2.

To facilitate development of an ovine contig spanning the predicted *clpg* interval, the ovine YAC library was further screened by PCR for the presence of OBDS562 and OBDS567, resulting in the identification of OY43H5 and OY403H1, respectively. High-density filters representing one segment of the RPCI-42 bovine BAC library were screened by hybridization for the presence of OBDS562, OBDS565, OBDS567, and *CKB.* This resulted in the identification of BAC clones 211A17 and 299O18 for OBDS562 and OBDS565, 208G17, 273E21, and 367B19 for OBDS567, and 322O10, 324D24, 463E13, 502H19, and 566A1 for *CKB.* Filters representing the entire bovine BAC library were screened with *YY1, AKT*, and *PREF-1* probes. Eight BACs containing *YY1* (6G20, 76P21, *211A17*, 299O18, 438A11, 476K21, 507E19, 545K5), 11 BACS containing *AKT* (74G15, 86A4, 86E2, 437F22, 478F13, 500A14, 519C14, 540F9, 551G13, 560B21, 560C20), and 10 BACS containing *PREF-1* (255M5, 275E24, 317J17, 320D23, 332L21, 367J11, 486B7, 525C4, 540H9, 549B15) were identified by hybridization and verified by PCR. Several MARC bovine EST sequences were found to have high sequence identity (>85%) with portions of the human *MEG3* gene. Probe corresponding to cDNA clone

1BOV13H14 (AW312758) was produced by random-prime labeling, hybridized to all isolated BAC clones, and found to reside on BACs 486B7 and 540H9.

Marker development. Large-insert genomic clones were subcloned by standard techniques (Sambrook et al. 1989). Microsatellites were identified by colony hybridization with end-labeled $(GT)_{11}$ oligonucleotide (Stone et al. 1995). Potential microsatellite clones were purified from bacterial cultures by alkaline lysis and sequenced for primer development. Single nucleotide polymorphisms associated with bovine *EMAPL, AKT,* and *BDKRB2* genes, as well as the ovine *AKT* and *EMAPL* genes, were identified by direct sequencing of PCR amplicons from sires of ovine resource (Freking et al. 1998a) and bovine reference populations (Bishop et al. 1994).

Linkage analysis. Marker genotypes were generated from the MARC bovine reference and ovine resource populations as described (Bishop et al. 1994; Freking et al. 1998a) and put into an interactive database (Keele et al. 1994). To order markers, genotypes for *EMAPL*, *PREF-1,* and *AKT* were established on a portion of the sheep resource population that had indications of recombination in the telomeric portion of OAR18. Linkage maps were constructed, and unlikely double recombinants were evaluated and genotypes reamplified when necessary. SNPs for the same locus were treated as a haplotype for multipoint linkage analysis. The linkage maps were constructed as described by Kappes et al. (1997) with Cri-Map version 2.4 (Green et al. 1990).

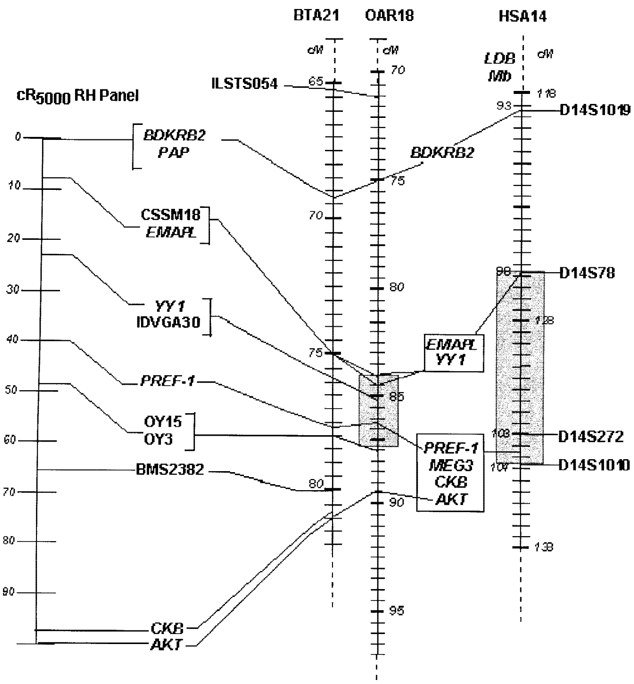

Fig. 1. A comparative genetic and H map of the bovine segment orthologous to the callipyge interval. *Bovine RH map:* RH map positions were determined with RHMAP3.0. The $cRAY_{5000}$ distance each marker is given relative to *PAP.* Type-1 markers are indicated in bold, type-2 are indicated in italics. *Bovine and Ovine Genetic Maps:* The meiotic map positions as predicted by Cri-Map version 2.4 are indicated. *Bovidae/Human comparative map:* The positions of type-2 markers common to the Marshfield meiotic and the Location Database (LDB) are indicated as (LDB/Marshfield). The position of type-1 markers predicted by the location database are 93.19 mb for *BDKRB2,* 98.047 for *EMAPL,* 97.977 for *YY1,* 103.54 for *PREF-1* and *CKB,* and 103.59 for *AKT.* The segment of HSA14 predicted to contain the *clpg* gene is indicated by a shaded box.

Results

The most efficient method to rapidly develop a physical map around the *clpg* locus is radiation hybrid mapping. Although no RH panel for sheep was available, the high conservation in sequence content and marker order between sheep and cattle (Cockett et al. 1994; de Gortari et al. 1997) suggested that the bovine RH panels would allow accurate preliminary estimations of the ovine physical map. Cell lines from the BovR5 RH panel were amplified with 10 primer pairs representing type-I (*CKB, PREF-1, PAP, BDKRB2, AKT, EMAPL,* and *YY1*) and type-II (IDVGA-30, BMS2382, and OBDS567) loci. These markers were selected based on their genetic proximity to the *clpg* locus, in the case of the type-II loci, or their presence on HSA14q24.3-31.1, in the case of the type-I loci. Retention frequencies for the markers tested were 13% for *EMAPL* and *YY1*, 14% for *CKB*, 16% for *PAP* and *BDKRB2*, and 17% for *PREF-1*, OBDS567, IDVGA-30, and BMS2382. Two-point analysis of the 5000-rad hybrid retention data for these 10 markers indicated all pairwise linkages among the 10 markers exceeded a LOD of 7.6 and supported a single linkage group with a LOD of 8.0. Multipoint analysis of these data (RHMAXLIK option RHMAP3.0) suggests that the marker order presented in Fig. 1 is 1.7 times more likely than any other order. Allowing for placement of *PREF-1* on either side of OY15, the order of the remaining markers is 8.4 times more likely than any other order, with a minimum number of 53 obligate breaks. The Centiray distance for these markers relative to the *PAP* locus is presented in Fig. 1.

The bovine RH panel analysis supported the conclusion that the telomeric region of HSA14 is orthologous to the region of OAR18 that harbors the *clpg* locus. To further support this conclusion, polymorphic markers for five of the genes which had been physically mapped using the RH panel were developed for genetic mapping in sheep and cattle. This approach had the advantages of integrating the physical and genetic maps of sheep, cattle, and humans, as well as addressing the possibility of rearrangement of the region owing to the *clpg* mutation. An ovine BAC that contained *BDKRB2* provided a microsatellite marker (#2355), as well as the sequence of the ovine *BDKRB2* promoter (Table 2). Primers were developed that amplified a portion of the *BDKRB2* promoter from cattle that contained two SNPs (haplotyped as marker #2473, Table 2) in the MARC bovine mapping families. The microsatellite marker #2355 was monomorphic in the MARC bovine reference parents but was polymorphic in the sheep resource population. Multipoint linkage analysis positioned *BDKRB2* between ILSTS054 and CSSM18 in both sheep and cattle (Tables 3 and 4, Fig. 1). The terminal intron of the bovine *AKT* gene was found to contain an SNP (marker #2272, Table 2) sufficient for preliminary mapping to the most telomeric position of the bovine meiotic map. *AKT* was subsequently mapped to 81 cM in cattle (Table 4) by virtue of a microsatellite (marker #2551) isolated from a bovine BAC containing the *AKT* gene. Several SNPs were also discovered in the terminal intron of the ovine *AKT* gene, but proved to be difficult to genotype. Ovine *AKT* was instead mapped to cM 89.4 (Table 3) by using the microsatellite marker #2697, also known as OarTRM1 (Robertson et al., submitted). In the course of bovine *AKT* BAC subcloning, a fragment with 84% sequence identity to three exons of the human KIAA0284 gene (AB006622) was discovered and found to be present on all bovine *AKT* BACs. This gene is known to reside on HSA14 and encodes a putative KARP-1-binding protein, potentially important to DNA double-strand break repair. We therefore concluded that the human ortholog of the gene underlying the callipyge phenotype lies between *BDKRB2* and *AKT/KIAA0284* on HSA14.

To determine whether any of the other type-I loci map to the interval containing *clpg* (CSSM18-OY15), the *EMAPL* gene was mapped (Tables 3 and 4) in both sheep (marker #2476) and cattle (marker #2412) by virtue of distinct deletions/insertions detected in an amplicon representing its 3′-end (Table 2). No recombinants between *EMAPL* and CSSM18 were detected with 10 co-informative meioses in sheep and 33 in cattle, suggesting their close genetic proximity. *CKB* was mapped in cattle (Table 4) by

Table 3. The number of recombinations unique to the smallest possible interval (below diagonal) and coinformative meiosis (above diagonal) for each pair of markers mapped to the ovine map. The diagonal represents the total number of informative meiosis for each marker.

	ILSTS054	MCM38	*BDKRB2*	CSSM18	*EMAPL*	IDVGA-30	*PREF-1/ MEG3*	OY3	OY5	OY15	*AKT*
ILSTS054	**1329**	1202	482	975	68	1054	114	1046	1091	1171	38
MCM38	9	**1683**	598	1337	71	1444	190	1390	1194	1546	42
BDKRB2	3	14	**675**	582	31	523	61	563	486	622	40
CSSM18	8	80	48	**1557**	10	1218	200	1235	1058	1424	43
EMAPL	0	8	6	0	**73**	69	4	73	68	71	12
IDVGA-30	1	18	5	6	0	**1513**	165	1250	1045	NA	NA
PREF-1/MEG3	0	0	0	1	0	8	**209**	181	125	201	36
OY3	0	14	0	13	1	16	8	**1613**	1185	1518	52
OY5	0	0	0	1	0	2	1	0	**1435**	1331	41
OY15	0	0	0	0	0	NA	0	0	0	**1773**	53
AKT	0	0	0	0	0	NA	0	0	0	1	**59**
(cM)[a]	71.5	72.2	75	84.1	84.5	85.2*	86.2	87.7	87.7	87.7	89.4

[a]The genetic position (cM = centimorgan) predicted upon linkage analysis with Cri-Map version 2.4.

Table 4. The number of recombinations unique to the smallest possible interval (below diagonal) and coinformative meiosis (above diagonal) for each pair of markers mapped to the bovine map. The diagonal represents the total number of informative meiosis for each marker.

	ILSTS054	*BDKRB2*	CSSM18	*EMAPL*	IDVGA-30	*PREF-1/ MEG3*	OY3	OY15	BMS2382	*CKB*	*AKT*
ILSTS054	**244**	147	91	34	135	203	129	133	78	191	165
BDKRB2	6	**219**	115	31	99	188	101	168	70	171	137
CSSM18	0	4	**176**	33	142	142	137	128	50	140	111
EMAPL	0	0	0	**34**	34	32	33	28	34	29	30
IDVGA-30	12	1	0	1	**206**	185	164	111	50	164	149
PREF-1/MEG3	0	5	0	0	2	**285**	168	178	82	232	192
OY3	0	0	0	0	0	1	**186**	105	45	158	145
OY15	1	2	0	0	0	0	0	**221**	70	176	137
BMS2382	0	0	0	0	0	0	0	4	**91**	70	46
CKB	1	0	0	0	0	0	1	2	1	**280**	197
AKT	0	0	0	0	1	0	0	0	0	1	**236**
(cM)[a]	65.2	69.3	75	75	75.8	77.5	77.9	77.9	80	80.6	81

[a]The genetic position (cM = Centimorgan) predicted upon linkage analysis with Cri-Map version 2.4.

virtue of a microsatellite (#2576, Table 2) isolated from a bovine BAC containing the *CKB* gene. As *CKB* mapped outside the interval of interest and very close to *AKT* in cattle, we did not map this gene in sheep. To map the *YY1* gene in cattle, the bovine BAC library was screened for clones harboring its 3′-end. Of the eight bovine BAC clones identified as containing *YY1,* clones 299O18 and 211A17 had been previously identified as containing IDVGA-30, indicating physical linkage between IDVGA-30 and *YY1.*

Bovine BACs were isolated that carry portions of both the *PREF-1* and *MEG3* genes, demonstrating their close physical proximity in the bovine genome. These two genes are also closely linked (91 kbp distance) in the human genome (overlapping BACs with GenBank accession numbers AL132711 and AL117190). Microsatellite markers isolated from these BACs (marker #2535 for cattle, #2575 for sheep, Table 2) were used to demonstrate that the *PREF-1* and *MEG3* lie within the highest likelihood interval containing the *clpg* locus (Freking et al, 1998a). This conclusion was based on recombinants between *PREF-1/MEG3* and IDVGA-30 (2 in cattle, 8 in sheep) or OY3/OY5/OY15 (1 in cattle, 9 in sheep). An assessment of the genotypes of the *PREF-1/MEG3* microsatellite in 209 ovine meioses (Table 4) reveals no inconsistencies between the phase of this marker and the *clpg* locus, assuming the polar overdominance model (Fig. 2). Therefore, *PREF-1* and *MEG-3* represent positional candidate genes for the *clpg* locus.

Discussion

The regulation of nutrient partitioning into protein and adipose tissues of meat animals is the subject of great interest. Some fat is important for normal metabolic function, but the diversion of feed into adipose production results in significant loss to meat producers (Keele and Fahrenkrug, submitted). In addition, consumers want low-fat, tender, and inexpensive meat products. The *clpg* gene provides an excellent opportunity to apply modern animal genetics to identify some of the factors important to these characteristics.

The genetic polymorphism that underlies the callipyge phenotype is yet to be determined. However, by identifying the segment of human Chr 14 orthologous to that containing the *clpg* locus, we have significantly narrowed the field of likely positional candidates. Assignment of *BDKRB2, EMAPL, YY1, PREF-1, CKB,* and *AKT* to ovine and/or bovine linkage maps allows integration of the bovine RH map with ovine and bovine genetic maps and supports the identification of a small segment of HSA14 extending from *YY1* to *AKT/KIAA0284* as being orthologous to the interval containing the *clpg* locus. At the present resolution, no large-scale rearrangements between species are apparent, with a conserved syntenic segment extending at a minimum from *BDKRB2* to *AKT.* These six genes have not been mapped in the highest resolution genetic map available for HSA14, the Marshfield map (Broman et al. 1998). However, they are predicted to lie between the framework markers D14S1019 and D14S1010 by the Location Database, LDB (Collins et al. 1996). Markers D14S1019 and D14S1010 lie within 15.6 cM of each other on the Marshfield genetic map of HSA14 (Broman et al. 1998), an interval similar in size to that predicted here for sheep and cattle, 14.4 cM and 11.8 cM, respectively. The interval estimated to contain the *clpg* locus in sheep is between CSSM18 and OY3/OY5/OY15 (3.9 cM), the most likely position being between IDVGA-30 and these OY markers (Freking et al. 1998a). Because we have demonstrated the physical proximity of *YY1* and IDVGA-30, the comparative interval is further refined to lie between *YY1* and *AKT.* This interval is 4.2 cM in sheep and 5.2 cM in cattle. Though *YY1* is not genetically mapped in humans, LDB predicts it to be very near the framework marker D14S78, reducing the size of the comparative interval in humans to 8.4 cM, the predicted distance between

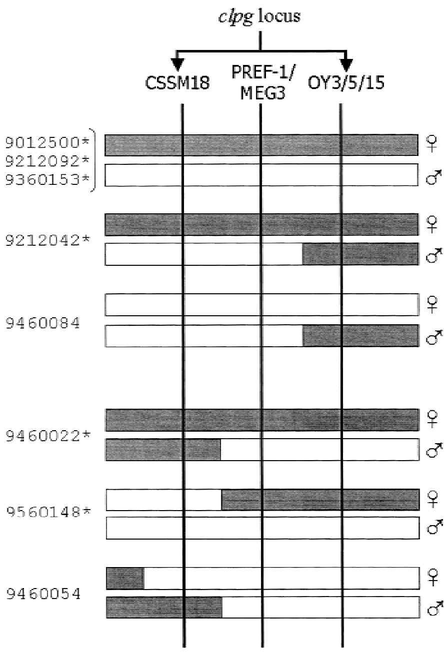

Fig. 2. The consistency of phase of *PREF-1/MEG3* alleles with the *clpg* locus. The phase of a mutant allele of the *clpg* gene is indicated by a white bar, while the phase of a normal *clpg* allele is indicated by a shaded bar. Animals displaying the callipyge phenotype are indicated by an asterisk (*). Progeny tested sires 9012500, 9212092, and 9360153 received maternal alleles for the indicated markers that are in phase with a normal *clpg* allele, and paternal alleles in phase with the mutant *clpg* alleles. Progeny 9212042, 9460084, 9460022, 9560148, and 9460054 display phase transitions between CSSM18 and OY3/5/15 (3.6 cM). All these animals displayed phenotypes consistent with the polar-overdominance model of the *clpg* locus. In each case the phase of *PREF-1/MEG3* is consistent with the phase of the *clpg* locus.

D14S78 and D14S1010 (Broman et al. 1998). Because the gene underlying the *clpg* locus is predicted to be about 3 cM centromeric of *AKT* in sheep, between IDVGA-30 and OY3/5/15, we reasoned candidates would be found in the telomeric-most half of the 8.4-cM comparative HSA14 interval. Two functional candidate genes fulfilling this criterion, *PREF-1* and *MEG3* were predicted to lie between D14S272 and D14S1010 by LDB, a 1.27-cM segment on the Marshfield map (Broman et al. 1998). We have mapped *PREF-1* and *MEG3* to the genetic interval suspected of harboring the *clpg* locus in sheep, as well as the orthologous DNA segment in cattle (Fahrenkrug et al. 1999). We consider *PREF-1* and *MEG3* excellent functional candidate genes for *clpg* based on their predicted function and/or expression profiles in other species.

Potential roles of both candidate genes in development of the *clpg* phenotype can be envisioned. *MEG3* transcription is known to be subject to imprinting (Schuster-Gossler et al. 1996, 1998), being expressed only from the maternal-derived chromosomes in both humans and mice (Miyoshi et al. 2000). In addition, expression is high in paraxial mesoderm of developing mice at a time coincident with myogenic differentiation. Therefore two important criteria for the *clpg* locus, imprinting and association with muscle development, are fulfilled by *MEG3*. However, since the *MEG3* transcript apparently does not encode a protein (Miyoshi et al, 2000, Schuster-Gossler et al, 1998), influence of this gene on the phenotype must invoke a functional role for the *MEG3* RNA.

Several features of the biology of *PREF-1* have led us to propose it as a candidate gene for *clpg*, in addition to its map position and phase consistency. *PREF-1* expression is down-regulated during adipocyte differentiation, and ectopic expression of Pref-1 inhibits adipogenesis (Smas and Sul 1993). These characteristics provide a potential mechanism for Pref-1 to affect fat accretion, one of the hallmarks of the callipyge phenotype. Pref-1 may also exert its effects on skeletal muscle indirectly, by stimulating the production of factors that influence muscle hypertrophy. In several species *PREF-1* is expressed in the adrenal gland and the pancreas during development, in adult adrenal medulla, a select population of cells in the adrenal cortex, and in insulin-producing beta-cells of the pancreas (Jensen et al. 1993, 1994; Tornehave et al. 1993, 1996). Pref-1 is likely to play a role in the development, differentiation, growth and function of the cells in these organs (Carlsson et al. 1997; Halder et al. 1998; Nielsen et al. 1999; Okamoto et al. 1998; Raza et al. 1998; Tornehave et al. 1996). The influence of catecholamines, corticoids and insulin products of these organs in nutrient partitioning and protein metabolism, as well as differentiation of both adipose and skeletal muscle, has been extensively documented (Allhaud et al. 1994; Ballard 1979; de Pablo et al. 1990; Heiman et al. 1999). However, to our knowledge the sensitivity of the expression of *PREF-1* to regulation by imprinting has not yet been examined.

It is also possible that both *PREF-1* and *MEG-3* genes play a role in mediating the *clpg* phenotype. The organization of *PREF-1/MEG3* locus is reminiscent of the *Igf2/H19* locus, a well-characterized example of reciprocal imprinting of tightly linked loci. At this locus, the maternally expressed *H19* tumor suppressor gene, which does not encode a protein, is located 90 kbp downstream from the paternally expressed insulin-like growth factor II (*IGF-II*) gene, and appears to influence the function of *IGF-II* by binding directly to an *IGF-II* binding protein (Runge et al, 2000). This suggests a possible model by which *MEG3* and *PREF-1* alleles could both be involved in development of the callipyge phenotype.

We have provided a genetic map of the ovine *clpg* locus capable of integrating comparative information from the human genome. In addition, we have described the linkage mapping of two specific genes, *PREF-1* and *MEG3,* to within the previously described interval containing the *clpg* locus. In 209 meiosis the phase of the *PREF-1/MEG3* marker was consistent with the *clpg* locus, assuming the polar overdominance model, lending support for these genes as positional candidates. While we consider the *PREF-1* and *MEG3* genes reasonable candidate genes for *clpg*, the comparative interval is likely to contain several positional/ functional candidate genes, each of which will require assessment by mapping and expression analysis in callipyge sheep. The comparative genetic map described here should prove valuable in narrowing the field of potential positional candidate genes to be subject to this type of analysis.

Acknowledgments. We greatly appreciate the collaboration of Eric Lord, Tracy Robertson, and Tom Broad at AgResearch, New Zealand, and Dr. Noelle Cockett, Utah State University. Helpful comments were provided by Tad Sonstegard and Zhanjiang Liu. Invaluable technical assistance was provided by Linda Flathman, Tracy Happold, Kevin Tennill, and Jeni Kuszak. We thank Sherry Kluver for assistance in the preparation of this manuscript.

Note added in proof: Schmidt et al. (2000) recently demonstrated mouse Pref-1 and Meg3 orthologs are reciprocally imprinted as proposed here (Genes & Dev. 14:1997–2002).

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